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Interleukin-2 (IL-2) has the capacity to activate lymphocytes to kill multidrug resistant cancer cells. Our phase I data established the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m2/day as a continuous i.v. infusion for 18 days) as consolidation treatment to patients with metastatic breast cancer early after intensive chemotherapy. Seven patients (60%) remained in complete remission at a median of >435 days post stem cell transplantation. We are therefore performing a phase II trial of AC+T chemotherapy followed by IL-2 consolidation (1 cycle as described above) in high risk stage II and III breast cancer patients. The goal is to kill residual chemotherapy-resistant cancer cells. Disease free survival and toxicity assessment represent major clinical aims (Specific aim 1). Immunologic effector mechanisms induced following MAT/SR by IL-2 infusion will be evaluated using phenotypic and functional assays for LAK cell induction (Specific Aim 2). Accrual to this study has been delayed due to a change from a randomized trial to a single arm phase II study. After overcoming regulatory and legal issues, the study finally opened 6/11/03. Ten patients been have accrued, 9 have completed planned treatment. Toxicity has been minimal to none. Two additional patients are being screened for enrollment. Laboratory correlation studies are proceeding and preliminary results demonstrate activation of circulating lymphokine-activated killer

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Introduction:

At least 46,000 women die from metastatic breast cancer each year in the United States. Median survival remains 12-18 months from the diagnosis of metastatic disease, and progression-free survival beyond 5 years is rare (<10%). This has led to the testing of escalated, marrow ablative doses of chemotherapy followed by stem cell rescue (MAT/SR). This approach produces a high frequency of objective responses in patients with metastatic breast cancer, with up to 40-50% complete responses. Unfortunately, responses tend to be short-lived, and only a minority of women (10-20%) achieve long-term disease free survival. Relapses may be due to both minimal tumor contamination of stem cells reinfused into patients, as well as residual chemotherapy resistant tumor cells not cleared by the MAT/SR regimen. IL-2 activated lymphocytes, termed lymphokine-activated killer (LAK) cells have significant cytotoxic activity against autologous breast cancer cells and breast cancer cell lines. Our own studies have demonstrated that multidrug-resistant tumor cells remain sensitive to LAK cell mediated killing. We have completed a phase I study to test the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m^2/day as a continuous i.v. infusion) as consolidation treatment to patients with metastatic breast cancer early after MAT/SR. This study established that IL-2 consolidation could be safely begun starting on day +14 post MAT/SR with minimal toxicity. Substantial LAK cell induction was observed, using flow cytometric and cytotoxicity assays. Thus far, only 3 of 10 patients have had breast cancer relapse or progression, and a small second breast cancer was detected in 1 patient. Seven patients (60%) remain in complete remission at a median of 435 days (range: 224 - 720 days) post stem cell transplantation. Because patients with metastatic breast cancer transplanted with active disease have a 60% and 80% probability of relapse at 1 year and 3 years, respectively (without IL-2), we proposed to test this promising immunotherapy consolidation strategy in a single-institution randomized prospective trial. We propose to perform cytoreduction in patients with metastatic breast cancer using MAT/SR, followed by continuous infusions of low-dose IL-2 starting on day +14 to activate lymphocytes to kill residual chemotherapy-resistant cancer cells. Based on preliminary data, we hypothesize that a single course of IL-2 will result in a significant improvement in disease-free survival, with minimal toxicity. Effectiveness of this approach may correlate with the effective induction of LAK precursor and effector cells, as well as evidence for reduction in the burden of minimal residual cancer cells. In **Specific Aim 1** we originally proposed to perform a prospective randomized clinical trial to test whether the addition of 1 cycle of continuous i.v. infusion of IL-2 in women with metastatic breast cancer, starting on day +14 after treatment with MAT/SR, can increase progression-free and overall survival by 25%. In **Specific Aim 2** we will evaluate possible immunologic effector mechanisms induced following MAT/SR and IL-2 infusion. Phenotypic and functional assays for LAK cell induction and enzyme immunoassays for circulating pro-inflammatory cytokines will be performed. Following review by the US Army, **Specific Aim 3** (detection of residual tumor cells in bone marrow and stem cell products by flow cytometry and RT-PCR) was omitted

Body:

Patient accrual to our proposed clinical trial has been severely delayed due to four unanticipated events. First, shortly after this proposal was funded in 1999, a series of randomized trials was reported at the American Society of Clinical Oncology meetings in 5/00 comparing standard dose chemotherapy and marrow ablative therapy and stem cell rescue (MAT/SCR) for treatment of advanced breast cancer. The conclusions of all but one of these trials was that there was no advantage to stem cell transplants in breast cancer patients over standard chemotherapy(1-3). The second event was that the one trial showing benefit of MAT/SCR over chemotherapy (Bezwoda, et al) was found to contain fraudulent data(4). In combination, these findings made the proposed control arm of our randomized clinical trial (MAT/SCR alone) unacceptable. Since the goal of MAT/SCR in our trial was to provide maximal cytoreduction prior to IL-2 based immunotherapy, this goal was still felt scientifically reasonable, given our impressive phase I trial results. In order to further prove the validity of these observations, it was felt by Dr. Peterson and myself that a change from a randomized trial to a single arm phase II study (MAT/SCR followed by an 18 day infusion of IL-2) was warranted. This change was discussed with the USAMRMC and the study protocol and consent documents were rewritten. A third point holding up the clinical trials was due to negotiations between the University of Utah lawyers and the USAMRMC concerning required liability clauses in the consent document and final approval by the University of Utah IRB. After many months of negotiations, a finalized consent language and protocol was agreed upon. A final draft has been submitted to the University of Utah IRB and was approved with minor revisions. A fourth event has necessitated further revisions in the study design. Further investigation into fraudulent data published by Bezwoda was presented at the ASCO meetings in 5/01. This resulted in the general abandonment of MAT/SCR as a breast cancer treatment modality in the United States. At the end of August of 2001, I was notified by my co-investigator Dr. Peterson that we would not be able to accrue any patients to a MAT/SCR regimen based trial, since patient referral to the University of Utah BMT program had dropped to nothing over the summer.

We have therefore reevaluated our options. Due to our exciting preliminary results, we still strongly believe that the concept of IL-2 consolidation in high-risk breast cancer should be tested after maximal cytoreduction. Given the apparent equivalence of MAT/SCR and standard chemotherapy in high risk breast cancer patients, we concluded that an alternative method to test our hypothesis is to enroll high-risk breast cancer patients who are treated systematically with surgery, followed by a standard chemotherapy regimen (doxorubicin/cyclophosphamide followed by paclitaxel or taxotere), followed by a 21 day infusion of IL-2. Patients will subsequently receive irradiation to the breast and regional node areas. Patients deemed at high risk include: patients with ≥4 lymph nodes positive (40% 5 year survival with >4+ nodes, <20% 5 year survival with >10 nodes involved), inflammatory breast cancer (<20% 5 year survival) and patients with resected stage IV disease (Stage IV NED, <10-20% 5 year survival)(5).

Dr. Peterson decreased his effort to 5% (since MAT/SCR will no longer be a primary treatment modality) and we added two Breast Cancer Medical Oncology specialists (Dr. John H. Ward and Dr. Saundra Buys), from the Huntsman Cancer Institute as co-

investigators to ensure adequate accrual. Since this trial is compatible with the community standard of breast cancer management in the Intermountain Region, the trial will be opened to Huntsman Cancer Institute affiliated community oncologists to increase the pace of accrual. It is envisioned that patient accrual for the proposed phase II trial (60 patients) can be completed in 36 months. The Huntsman Cancer Institute Clinical Trials Office will provide the resources for the long-term follow-up patients beyond the scope of the current grant funding. This revised protocol has been completed and has been approved by the University of Utah IRB and by the US Army Medical Research and Material Command (HSRRB Log Number A-9034). A final amendment was incorportated due to changes in breast cancer treatment practice patterns, allowing use of adriamycin and cytoxan with either Taxol or Taxotere. These drug combinations could be given in sequence or concomitantly, since current evidence suggests equivalence. The protocol finally opened to patient accrual 6/11/03. Ten patients have been enrolled, and 9 have completed planned therapy. Toxicity to date has been minimal to none (no toxicity > grade 1 noted). One patient had a brief interruption in treatment due to fever, which subsided and the patient was able to complete planned treatment. No patient enrolled on the study has experienced a cancer relapse. Two additional patients are being screened for enrollment currently. Clinical samples have been submitted to the laboratory for testing. Preliminary results indicate induction of circulating LAK cells in all patients, albeit with moderate variability in maximum cytotoxicity at 100:1 effector to target cell ratio. It will be interesting to see if this appears to correlates with eventual treatment outcome and progression-free survival. Our proposal is to complete patient accrual over 3 years, due to the delayed start of the trial, as a no-cost extension, using funds carried over from preceding years (with additional support from the Huntsman Cancer Institute).

Task 1: Patient Enrollment: (months 1-36-start delayed to month 36)

- Protocol will be presented to eligible patients prior to chemotherapy for high risk breast cancer. Eligible patients will include ≥4+ nodes, inflammatory breast cancer and completely resected stage IV disease. All patients will be treated with at least 4 cycles of doxorubicin/cyclophosphamide and 4 cycles of paclitaxel chemotherapy (AC+T) at 3-4 week intervals.
- Appropriate people/departments will be notified of patient enrollment and randomization, to include: site pharmacy, Dr. Wolf Samlowski and/or lab and Dr. Wayne Green and/or lab.
- A Progress Note will be entered into the patient's medical record regarding patient consent, enrollment, randomization, and the study requirements.

Task 2: Administration of IL-2: (months 1-36 from revised start date)

- On Day + 14 following completion of 4 cycles of AC+T, the patient will come to clinic (if discharged), where they will be have their vital signs taken and be evaluated by a physician or physician extender for a baseline physical exam.
- The CADD-1 pump and supplies will be reviewed with the patient and caregiver(s).
- IL-2 will be started and the patient will remain in clinic for at least one hour to monitor vital signs and any adverse reactions.
- A patient diary will be given to the patient to help monitor and track fevers, other reactions, admissions, etc. (See attached sample.)
- Patient will be seen in clinic a minimum of once per week and also as needed. IL-2 cassettes will be changed every six days by the Research Nurse. Review of any adverse reactions or other problems will take place.

Task 3: Specimen Collection: (months 1-44 from revised start date)

- Approximately 50 cc's of blood will be collected in heparinized, green top tubes on Day 0, +7, + 14, +21, +32 and +100 and delivered to Dr. W. Samlowski's and Dr. W. Green's labs. (See below for details of lab procedures)
- Samples of pre-transplant and day 100 bone marrow material, as well as stem cell products will be transported to Dr. W. Samlowski's lab for evaluation of minimal residual tumor cells (5 ml marrow or PBSC cells in a heparinized syringe.

Task 4: Analysis of LAK cell induction (months 1-44 from revised start date)

- Samples will be analyzed for T cell and LAK cell markers by flow cytometry (Dr. Green's lab)
- Analysis of patient samples for LAK precursor and cytolytic cell function will be performed (Dr. Samlowski's lab)

Task 5: Assays for tumor cell detection in bone marrow and stem cell products (deleted from funding by USAMRMC)

Task 6: Data Collection (months 1-48 from revised start date):

- The following will be collected: dates of each chemotherapy cycle and treatment doses, and side effects. While patients are receiving IL-2 infusions, side effects of

IL-2 (fevers, rash, etc), infections, readmission, relapse, death and other significant events will be recorded.

- At day 100, the patient will be seen by the physician to evaluate their disease and health status. Information such as infections, readmission, relapse and death will be collected.
- Patients will then be followed at least every 3 months or as needed to monitor disease status and/or death.

Task 7: Interim Analysis: (approximately month 24 from revised start date)

- After approximately 30 patients are enrolled, data collected from Dr. Wolf Samlowski's lab and Dr. Wayne Green's lab, together with information collected in the CRF's will be analyzed by the principal investigators.

Task 8: Final Analysis: (month 60 from revised start date)

- After enrollment of 60 patients is complete, data collected from Dr. Wolf Samlowski's lab and Dr. Wayne Green's lab and information collected in the CRF's will be analyzed by the principal investigators. At the completion of this study a report will be generated.

Key research accomplishments:

We treated 20 patients with MAT/SCR in our phase I pilot trial. Patients received IL-2 either starting on day 1 (10 patients) or day 14 (10 patients) following stem cell infusion. A total of 17 patients were evaluable for response at the time of initial analysis. A total of 17 patients (85%) completed the IL-2 course. Three patients receiving IL-2 from day 1 required IL-2 infusions to be terminated early (2 fever, 1 thrombocytopenia). Relapse free survival was 45% with 580 day median follow-up (135-1175 days), with 75% overall survival.

LAK cell activation was evaluated in patients undergoing IL-2 infusions starting either day 1 (5 patients) or day 14 post stem cell infusion (5 patients). Cytotoxicity against the MCF-7 breast cancer line was detected in all patients, regardless of whether the IL-2 infusion was started day 1 or 14. Increased cytolytic activity was detected in cytotoxicity assays performed with the addition of IL-2, suggesting a substantial increase in circulating LAK cell precursors in both patient populations. Phenotypic evaluation established that while CD56+ cell populations were expanded in both patient groups, the absolute number of circulating CD56+ cells was 10-fold higher in patients receiving IL-2 starting on day 14.

Due to these results, our current clinical trial will treat patients beginning on day +14 after completion of 4 cycles of AC and 4 cycles of Taxol with a 18 day infusion of IL-2 to verify these exciting clinical results in this high-risk breast cancer population. Three patients have been entered to date. Treatment related toxicity has been negligible (none > grade 1). There has been no disease progression to date (follow up too short).

Reportable outcomes:

Abstract presented at Era of Hope Meeting 9/25/02-9/28/02 (enclosed)

Petersen FB and **Samlowski WE.** Feasibility of low dose continuous infusion of IL-2as a consolidation treatment following intensive breast cancer chemotherapy. Proceedings Era of Hope DOD Breast Cancer Research Program Mtg. 2002, p 33-20

Conclusions:

The proposed use of IL-2 following maximal cytoreduction of tumor by standard chemotherapy or MAT/SCR remains promising based on our preliminary data, with 45% of patients achieving >2year disease free survival after MAT/SCR plus IL-2. We have started a phase II pilot study to evaluate the effectiveness of this regimen with standard adjuvant chemotherapy in high-risk breast cancer patients in a phase II trial. Preliminary data shows toxicity to be minimal and feasibility of immunologic monitoring.

References:

1) Stadtmauer EA et al. Phase III randomized trial of high dose chemotherapy and stem cell support shows no difference in overall survival or severe toxicity compared to maintenance chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) for women with metastatic breast cancer who are responding to standard induction chemotherapy Proc ASCO 18:1a, 1999.

2) Peters W, et al. A prospective randomized comparison of two doses of combined alkylating agents as consolidation after CAF in high-risk primary breast cancer involving ten or more axillary lymph nodes: Preliminary results of CALGB 9082/SWOG9114/NCIC MA-13. Proc ASCO 18:1a, 1999.

3) Scandinavian Breast Cancer Study Group. Results of a randomized adjuvant breast cancer study with CTCb supported by autologous bone marrow stem cells versus dose-escalated and tailored FEC therapy. Proc ASCO 18:2a, 1999.

4) Bezwoda WR. Randomized controlled trial of high dose chemotherapy (HD-CNVp) versus standard dose (CAF) chemotherapy for high-risk, surgically treated primary breast cancer. Proc ASCO 18:2a, 1999.

5) Donnegan WL. Staging and primary treatment. In Donegan WL, Spratt JS eds, Cancer of the Breast. W. B. Saunders, 1998, pp 336-402.